Serum and vitreous humor antibody titers in and isolation of *Leptospira interrogans* from horses with recurrent uveitis

Bettina Wollanke, Dr med vet; Barton W. Rohrbach, VMD, MPH, DACVPM; Hartmut Gerhards, Prof Dr med vet

**Objective**—To measure antibody titers against *Leptospira interrogans* in serum and vitreous humor and determine the prevalence of *L. interrogans* in vitreous humor of horses with recurrent uveitis.

**Design**—Cross-sectional study.

**Animals**—242 horses (270 eyes) with recurrent uveitis undergoing vitrectomy and 39 control horses (54 eyes) without any history or clinical signs of recurrent uveitis undergoing euthanasia or enucleation for unrelated reasons.

**Procedure**—Serum and vitreous humor were tested for antibodies against 13 serovars of *L. interrogans*. Vitreous humor was submitted for leptospiral culture; isolates were typed to the serogroup level.

**Results**—*Leptospira interrogans* was isolated from vitreous humor from 120/229 (52%) horses (126/252 [50%] eyes) with recurrent uveitis but was not isolated from vitreous humor from 36 eyes of 21 control horses. Duration of recurrent uveitis was ≥ 1 year for 45 of the 120 (38%) horses from which the organism was isolated. Geometric mean antibody titers against *L. interrogans* in the vitreous humor and serum of horses with recurrent uveitis were 1:1,332 and 1:186, respectively. Only 91 of 120 (76%) horses from which the organism was isolated had a 4-fold or greater difference between serum and vitreous humor antibody titers.

**Conclusions and Clinical Relevance**—Results suggest that persistent ocular infection with *L. interrogans* is common in horses with recurrent uveitis. A 4-fold increase in vitreous humor versus serum antibody titers may not be a sensitive test for the diagnosis of *L. interrogans*-induced recurrent uveitis. We hypothesize that the immune component of recurrent uveitis can be directly induced and maintained by persistent infection of the eye with *L. interrogans*. (J Am Vet Med Assoc 2001;219:795-800)

Numerous theories have been advanced to explain the development of recurrent uveitis in horses. In most clinical cases, an etiologic agent cannot be identified. However, in the 1940s, serum antibody titers against *Leptospira interrogans* were found to be higher in horses with recurrent uveitis than in healthy control horses, leading to speculation that infection with *L. interrogans* was causally associated with recurrent uveitis. Since then, numerous studies have identified antibodies against *L. interrogans* in serum and ocular fluid from horses with recurrent uveitis. Authors of 1 study, however, concluded that infection with *L. interrogans* did not play a major role in the development of recurrent uveitis, because the prevalence of serum antibody titers against *L. interrogans* in horses with recurrent uveitis (9%) was similar to that in healthy horses (11%).

Experimental infection of horses with *L. interrogans* serovar pomona and natural outbreaks of leptospirosis have been associated with recurrent uveitis, although most adult horses do not develop recurrent uveitis until 12 to 24 months after experimental infection. Similarly, human patients may develop uveitis in association with leptospiral infection, although they typically do so during the acute phase of infection or within the first months after infection. Several reports describe isolation of *L. interrogans* from the ocular fluid of horses with recurrent uveitis and leptospiral DNA was recently identified in the aqueous humor of 21 of 30 (70%) horses with clinical recurrent uveitis and 1 of 16 horses without uveitis. Studies on the mechanism of inflammation in recurrent uveitis have shown that antibodies against *L. interrogans* will bind to the equine cornea and lens in vivo. In addition, the third component of complement was found to bind to equine corneal cells when incubated with anti-leptospiral serum in vitro, and the authors hypothesized that antigenic cross-reactivity, in addition to infiltration of the cornea with neutrophils and lymphocytes, might explain the tissue damage in horses with recurrent uveitis. More recently, a local direct antibacterial immune response induced and maintained by persistent leptospiral infection and an autoimmune response against intraocular tissue were proposed as possible mechanisms of ocular inflammation in horses with recurrent uveitis, and a search for *L. interrogans* or its antigens in intraocular tissue was proposed.

The purpose of the study reported here was to evaluate the hypotheses that *L. interrogans* is a common cause of recurrent uveitis in horses and that persistent ocular infection may be responsible for recurrence of uveitis in horses.

**Materials and Methods**

**Horses**—Two hundred forty-two horses in which a clinical diagnosis of recurrent uveitis had been made were used in the study. Information for 104 of the 242 horses has been published previously. In all horses, the diagnosis of recurrent uveitis had been made by the referring veterinarian and confirmed by a faculty member (BW or HG) of the University of Tennessee, Knoxville, TN 37901 (Rohrbach).

The authors thank Drs. Siegfried Brem, Hartmut Kopp, and Peter Meyer for technical assistance.
of Munich. All horses were examined in a darkened room, using a focal light source and magnification when necessary. The eyelids, conjunctiva, cornea, anterior chamber, anterior lens capsule, and lens were examined, and pupillary light responses were evaluated. In horses with transparent or semitransparent lenses, the posterior lens capsule, vitreous humor, and fundus (including the retina) were examined with an ophthalmoscope.

A diagnosis of recurrent uveitis was made if horses had a history of at least 2 episodes of anterior or posterior uveitis or had clinical signs of uveitis, including 1 or more of the following: atrophy of the ocular bulb and a history of uveitis, inflammatory products in the anterior chamber without signs of trauma, inflammatory products on the posterior lens capsule, cataracts secondary to posterior synechiae or inflammatory products on the posterior lens capsule, cataracts secondary to synechia or inflammatory products in the vitreous humor and on the posterior lens capsule (verified by means of ultrasonography, if necessary), inflammatory products causing diffuse opacity or forming clots in the vitreous humor, or partial or complete retinal detachment or choroidal scars with signs of inflammation of the vitreous humor. Ocular changes varied from few signs of inflammation to blindness.

Thirty-nine control horses (54 eyes) were also included in the study. Control horses were selected from horses admitted to the veterinary teaching hospital between June 1994 and March 2000 for euthanasia or enucleation for reasons other than recurrent uveitis. Control horses underwent an ophthalmic examination and did not have any visible evidence of recurrent uveitis.

Sample collection and handling—Serum and vitreous humor were obtained from the horses at the time of vitrectomy, enucleation, or euthanasia. For the horses with recurrent uveitis, vitreous humor was obtained at the time of vitrectomy, enucleation, or euthanasia. For the horses with recurrent uveitis to examination at the veterinary teaching hospital ranged from 4 weeks to several years; 115 of 241 (48%) horses had a history of recurrent uveitis of ≥ 1 year’s duration. Median age of horses with recurrent uveitis (6 years; range, < 1 to 22 years) was not significantly different from median age of the control horses (7 years; range, < 1 to 27 years). Similarly, sex distribution of the horses with recurrent uveitis (101 females, 115 geldings, 19 stallions) was not significantly different from that of the control horses (18 females, 13 geldings, 5 stallions).

Serum antibody titers against \( L \text{ interr} \) gans serovars grippotyphosa, copenhageni, bratislava, canicola, sawkoebing, hardjo, tarassovi, pyrogens, javanica, pomona, sejroe, hebdomadis, and autumnalis in the serum and vitreous humor samples were determined by use of a microagglutination test (MAT).\(^6\) The serovar against which antibodies in serum and vitreous humor were directed was identified as the serovar for which the titer was the highest. If the highest titer was shared by 2 or more serovars or if identified as the serovar for which the titer was the highest.

Serum and vitreous humor antibody titers were evaluated. In horses with bilateral recurrent uveitis, information for the eye from which \( L \text{ interr} \) gans was isolated was used for statistical analyses. If \( L \text{ interr} \) gans was isolated from both eyes or from neither eye, the eye used for statistical analyses was randomly chosen. For control horses in which both eyes were enucleated, the eye with the highest titer for antibodies against \( L \text{ interr} \) gans in the vitreous humor was used; if neither eye had a titer for antibodies against \( L \text{ interr} \) gans, the eye used for statistical analyses was randomly chosen.

The \( \chi^2 \) test was used to compare values for categorical data (eg, sex, whether clinical signs had been evident for ≥ 1 year, antibody titer ≥ 1:400 in serum or vitreous humor, 4-fold increase in antibody titer between serum and vitreous humor, and proportion of horses with antibody titer against a specific serovar and proportion of horses with isolation of a specific \( \text{Leptospira} \) serogroup), and Student t-tests or Wilcoxon 2-sample tests were used to compare values for continuous data (eg, age, log transformed values for antibody titers in serum and vitreous humor) among and within subgroups (eg, horses with a clinical diagnosis of recurrent uveitis vs control horses and horses with recurrent uveitis from with \( L \text{ interr} \) gans was isolated vs horses with recurrent uveitis from which \( L \text{ interr} \) gans was not isolated). Antibody titers were logarithmically transformed (base 10) prior to analysis, and mean values were back-transformed to obtain geometric mean titers (GMT). Adjustments for multiple comparisons were performed, using the technique of Bonferroni.\(^7\) Statistical analyses were performed with computer software.\(^7\) Values of \( P \leq 0.05 \) were considered significant.

Results

In total, 270 eyes were affected in the 242 horses with recurrent uveitis. Time from the initial episode of recurrent uveitis to examination at the veterinary teaching hospital ranged from 4 weeks to several years; 115 of 241 (48%) horses had a history of recurrent uveitis of ≥ 1 year’s duration. Median age of horses with recurrent uveitis (6 years; range, < 1 to 22 years) was not significantly different from median age of the control horses (7 years; range, < 1 to 27 years). Similarly, sex distribution of the horses with recurrent uveitis (101 females, 115 geldings, 19 stallions) was not significantly different from that of the control horses (18 females, 13 geldings, 5 stallions).

Serum antibody titers against \( L \text{ interr} \) gans serovars were obtained for 241 of the 242 horses with recurrent uveitis and for 37 of the 39 control horses. Geometric mean serum antibody titer for the horses with recurrent uveitis (1:186) was significantly \((P < 0.001)\) higher than mean titer for the control horses (1:64). Serum antibody titers ≥ 1:400 against 1 or more serovars of \( L \text{ interr} \) gans were found in 106 of 241 (44%) horses with recurrent uveitis but in only 7 of 37 (19%) control horses; these percentages were significantly \((P < 0.004)\) different.

Geometric mean vitreous humor antibody titer for the horses with recurrent uveitis (1:1,332) was also significantly \((P < 0.001)\) higher than mean titer for the control horses (1:1). Vitreous humor antibody titers ≥ 1:400 against 1 or more serovars of \( L \text{ interr} \) gans were found in 194 of 242 (80%) horses with recurrent uveitis but were not found in any of the 39 control horses; these percentages were significantly \((P < 0.003)\) different. Only 2 control horses had vitreous humor antibody titers against \( L \text{ interr} \) gans; for both horses, the titer was 1:100.
Vitreous humor samples from 252 eyes of 229 horses with recurrent uveitis and from 36 eyes of 21 control horses were submitted for bacterial culture. Leptospires were isolated from 126 of the 252 (50%) eyes (120/229 [52%] horses) with recurrent uveitis but were not isolated from any of the 36 control eyes. Typing to the serogroup level was available for 110 of the 126 isolates (105/120 horses with recurrent uveitis), and grippotyphosa was the serogroup isolated most frequently (Table 1). For 88 of 100 (88%) horses, the serovar against which antibodies in the vitreous humor were directed matched the serogroup isolated by means of bacterial culture. Similarly, for 52 of 79 (66%) horses, the serovar against which serum antibodies were directed matched the serogroup isolated by means of bacterial culture.

The serovar against which antibodies in the vitreous humor were directed could be identified for 204 of the 242 horses with recurrent uveitis, and the serovar against which antibodies in the serum were directed could be determined for 171 horses (Table 2). The serovar against which antibodies in the serum were directed could be determined for 22 of the 39 control horses. Grippotyphosa was the serovar against which vitreous humor and serum antibodies were most commonly directed in horses with recurrent uveitis, whereas bratislava was the serovar against which serum antibodies were most commonly directed in control horses. The only 2 control horses with vitreous humor antibody titers (1:100) had antibodies directed against serovar grippotyphosa. For horses with recurrent uveitis, the proportion that had vitreous humor antibody titers against serovar grippotyphosa (79%) was significantly higher than the proportion that had serum antibody titers against this serovar (44%), and the proportion that had vitreous humor antibody titers against serovar bratislava (8%) was significantly lower than the proportion that had serum antibody titers against this serovar (22%). Proportions of horses with recurrent uveitis and control horses that had serum antibody titers against grippotyphosa and against bratislava were not significantly different.

For horses with recurrent uveitis, those for which L. interrogans was isolated from the vitreous humor did not differ significantly from those for which L. interrogans was not isolated in regard to age, sex, or geometric mean serum antibody titer (Table 3). However, geometric mean vitreous humor antibody titer was significantly higher for horses from which organisms were isolated than from horses for which results of bacterial culture were negative. The percentage of horses with a 4-fold difference between serum and vitreous humor antibody titers was significantly higher for horses from which organisms were isolated than from horses for which results of bacterial culture were negative. On the other hand, the percentage of horses with clinical signs for ≥ 1 year was significantly higher for horses for which results of bacterial culture were negative, compared with horses from which organisms were isolated.

Proportions of horses with recurrent uveitis for which vitreous humor antibody titers were directed against each of the serovars of L. interrogans were remarkably similar between horses from which organ-
isms were isolated and horses for which results of bacterial culture were negative (Table 4). Greater variability was seen when proportions for which serum antibody titers against each of the serovars of *L. interrogans* were compared between these groups.

### Discussion

Results of the present study support the hypothesis that persistent leptospiral infection has an important role in the pathogenesis of recurrent uveitis in horses. Leptospires were isolated from vitreous humor samples from 120 of 229 (52%) horses with recurrent uveitis, and signs had persisted for ≥ 1 year in 45 of the 120 (38%) horses from which organisms were isolated. In addition, the geometric mean vitreous humor antibody titer for horses with recurrent uveitis from which *L. interrogans* was isolated was significantly higher than that for horses with recurrent uveitis for which results of bacterial culture were negative. However, proportions of horses in which vitreous humor antibodies were directed against each serovar were remarkably similar between horses from which the organisms were isolated and horses for which results of bacterial culture were negative. Because *L. interrogans* is a fastidious organism and difficult to isolate in culture, we suspect that some horses with recurrent uveitis for which results of bacterial culture of vitreous humor were negative actually were harboring the organism but in numbers too low to be detected by current culture techniques. Therefore, the percentage of horses with recurrent uveitis that also had leptospiral infection may have been underestimated in this study. This would agree with results of a recent study in which leptospiral DNA was identified in 21 of 30 (70%) horses with recurrent uveitis, but *L. interrogans* was isolated from only 6 (29%).

Although the percentage of horses with recurrent uveitis that had serum antibody titers ≥ 1:400 (106/241; 44%) was significantly higher than the percentage of control horses that did (7/37; 19%), measurement of serum antibody titers appears to be of little value in the diagnosis of recurrent uveitis. In contrast, we found that 194 of 242 (80%) horses with recurrent uveitis had vitreous humor antibody titers ≥ 1:400, whereas none of the 39 control horses did.

Of 241 horses with recurrent uveitis, 151 (63%) had a 4-fold or greater difference between vitreous humor and serum antibody titers. In addition, 22 of the 241 (9%) horses, including 8 of 120 (7%) horses from which *L. interrogans* was isolated, had serum titers less than the lowest dilution tested (1:100), whereas the vitreous humor antibody titers for the 8 seronegative horses from which the organism was isolated ranged from 1:400 to 1:51,200. Finally, the serovar against which serum antibodies were directed was different from the serovar against which vitreous humor antibodies were directed in 58 of 144 (40%) horses with recurrent uveitis, including 35 of 85 (41%) horses from which *L. interrogans* was isolated. Taken together, these findings suggest that antibodies in the vitreous humor are likely a result of local production and not a result of antibody transfer through the blood-ocular barrier.

A 4-fold or greater difference between serum and vitreous humor antibody titers was found in 91 of 120 (76%) horses with recurrent uveitis from which the organism was isolated but was also found in 53 of 109 (49%) horses with recurrent uveitis for which results of bacterial culture were negative. These data indicate that using a 4-fold difference between serum and vitreous humor antibody titers to identify horses with ocular *L. interrogans* infection has low sensitivity and specificity and do not support use of this test to identify horses with leptospiral-induced recurrent uveitis. The low test specificity may have been attributable to an inability to isolate *L. interrogans* from infected horses with low numbers of the organism in the vitreous humor, which led to misclassification of these horses as not being infected.

In the present study, the serovar with the highest vitreous humor antibody titer matched the serogroup obtained by means of bacterial culture in 88 of 100 (88%) horses. This suggests that intraocular infection with *L. interrogans* is the principal stimulus for antibody production, although it does not rule out the possibility that antibody production could be an autoimmune response directed against ocular tissue. Two of the 39 (5%) control horses, which did not have a history or clinical signs of recurrent uveitis, had vitreous humor antibody titers at the lowest dilution tested (1:100) against serovar grippotyphosa. One of these

### Table 4—Numbers of horses with recurrent uveitis from which *L. interrogans* was or was not isolated from vitreous humor that had serum and vitreous humor antibodies against various serovars of the organism

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Leptospires isolated (n = 89)</th>
<th>Leptospires not isolated (n = 73)</th>
<th>Leptospires isolated (n = 112)</th>
<th>Leptospires not isolated (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grippotyphosa</td>
<td>46 (52)</td>
<td>24 (33)</td>
<td>89 (79)</td>
<td>64 (79)</td>
</tr>
<tr>
<td>Bratislava</td>
<td>22 (25)</td>
<td>14 (19)</td>
<td>10 (9)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Copenhageni</td>
<td>10 (11)</td>
<td>16 (22)</td>
<td>3 (4)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Saxkoebing</td>
<td>1 (1)</td>
<td>5 (7)</td>
<td>1 (1)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Hardjo</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Pyrogenes</td>
<td>6 (7)</td>
<td>10 (14)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Pomona</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tarassovi</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Canicola</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are given as No. of horses (%). Horses were considered to have antibodies against a particular serovar if titer for that serovar was the highest titer for all 13 serovars tested.
horses had a serum antibody titer against serovar grippotyphosa of 1:400, and the other was seronegative (<1:100). It is possible that these horses may develop recurrent uveitis in the future.

*Leptospira interrogans* was isolated from 120 of 229 (52%) horses with recurrent uveitis in the present study. For 32 of 37 (86%) horses from which leptospires were isolated in which duration of clinical signs was ≥1 year, the serovar against which vitreous humor antibodies was directed matched the serogroup obtained by means of bacterial culture. Isolation of *L. interrogans* from horses with chronic recurrent uveitis suggests that antibody titers against *L. interrogans* in the vitreous humor can be induced and maintained by leptospires within the eye. This would support the hypothesis that the pathogenesis of recurrent uveitis involves a combination of direct bacterial effects and the effects of locally produced antibodies against *L. interrogans* that cross-react with ocular tissue. However, if direct bacterial effects do play an important role in the inflammation associated with recurrent uveitis, it is not clear why potent immunosuppression with corticosteroids would have a beneficial effect.

If the primary mechanism of inflammation in recurrent uveitis was an autoimmune response against intraocular tissue, and the presence of *L. interrogans* was not necessary for maintenance of antibody production, one would not expect vitrectomy to eliminate recurrences of uveitis. However, in almost all cases, additional episodes of uveitis can be eliminated by removing the vitreous humor and flushing the vitreous humor chamber with antibiotics (20 mg of gentamicin in 250 ml of a balanced salt solution). Because ocular proteins remain after this procedure, one would expect the episodes to recur if an autoimmune response mediated by T or B memory cells against these antigens were the primary mechanism of inflammation.

In conclusion, results of the present study suggest that infection with *L. interrogans* is a more common cause of recurrent uveitis in horses than has been previously recognized. We hypothesize that persistence of leptospiral infection in the vitreous humor of horses with recurrent uveitis can induce and maintain an autoimmune response. During the period between episodes of uveitis, the number of leptospiral organisms may become too low to be detected with current bacterial culture techniques, and antibody titers may decrease sufficiently so that any inflammatory response in the eye is undetectable clinically. When the antibody titer decreases below a threshold or the immune system is compromised, the organisms may reactivate, causing direct bacterial effects that, in combination with an increase in antibodies against *L. interrogans* that cross-react with ocular tissues, induces the inflammatory response typical of recurrent uveitis. As a corollary, we hypothesize that the immune component of recurrent uveitis is directly induced and maintained by persistent leptospiral infection of the eye.

References


**Correction: Risk factors associated with development of postoperative ileus in horses**

In the report “Risk factors associated with development of postoperative ileus in horses” (*JAVMA*, Jul, 2001, pp 72–78), there were several omissions in Table 1. In the first column, under the heading “Variable,” the fourth, fifth, sixth, and seventh variables should read “Serum total protein (per g/dl),” “Serum albumin (per g/dl),” “Duration of anesthesia/h,” and “Duration of surgery/h,” respectively. The heading of the third column should read “Horses without POI (No. [%]),” and the heading of the fourth column should read “Odds ratio (95% CI).”